

Appl. No. 10/665,105  
Amdt. Dated 03/08/2006  
Reply to Office action of November 8, 2005

**Amendments to the Drawings:**

The attached sheet of drawings includes changes to Fig. 1. This sheet, which includes Fig. 1, replaces the original sheet including Fig. 1. In Figure 1, previously omitted element 20 has been added.

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### REMARKS/ARGUMENTS

Status

Claims 1-32 are pending

Claims 13-32 are withdrawn from consideration

Claims 1-12 are rejected

The Office Action stated that the disclosure was objected to because of the following informalities: what should presumably be the chemical name gallocyanide on p.5, I. 11 and p. 9, I. 14 is misspelled. This has been corrected.

The Office Action stated that the specification should be revised carefully in order to comply with 35 U.S.C. 112, first paragraph. Examples of some unclear, inexact or verbose terms used in the specification are: the description of Fig. 5 (p. 12, 1.27 to p. 13, 1.7). Applicant refers to unspecified samples being taken from hitherto unmentioned herds of animals, resulting in percentages of false positives of some test that is completely unspecified. The description is lacking in sufficient detail to enable the reader to understand what is being carried out in this example. An amendment to the specification, as set forth below, has been proffered in the present response to the Office Action. It is believed that this amendment should clear up any lacking in sufficient detail so as to enable the reader to understand what is being carried out in this example.

Here is the amended paragraph with the additional language underlined. beginning on page 12, line 26 to page 13, line 7.

"Referring to Figure 5, there are two sets of examples from two different herds, each of 100 animals. Using the prior art, typical pressure analysis of a mixture of a culture broth and reagents and a sample from each animal, the percentage of false positives for the first herd was 27.14% and for the second herd was 6.33%, where there was actually no organism. The samples that

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were mixed with the culture broth and reagents and placed into the bottle were processed fecal samples that may contain "mycobacterium paratuberculosis. Using the pressure analysis of a mixture of a culture broth and reagents and two poisoning agents, in accordance with the present invention, and a sample from each animal, the percentage of false positives for the first herd was reduced to 5.71%, and for the second herd was reduced to 0.00%, where there actually was no organism."

The Office Action stated that the drawings were objected to under 37 CFR I .83(a) because they fail to show the vial headspace 20 (Fig. 1 ) as described in the specification. An amended replacement drawing sheet with Figure 1 is submitted wherein previously omitted element 20 has been added and with "New Sheet" labeled in the top margin.

In the Office Action, Claims 1-12 were rejected under 35 U.S.C. 103(a) as being unpatentable over Eden et al. [US 5,232,839] in view of Lancaster et al. [US5,501,839].

The Office Action characterized Claims 1-12 as being drawn to a method of stabilizing the output signal of a system that detects microbiological growth in a sealed container, comprising the steps of i) providing a sealed container containing a culture broth, the sample and at least one poisoning agent, ii) monitoring pressure changes within the head space of the sealed sample container, and iii) indicating a presence of microbiological growth within the sealed sample container as a function of the change in the headspace pressure (claim 1). The Office Action went on to state that the method was further specified to comprise providing a pair of coupled poisoning agents (claim 2) selected from the group consisting essentially of ferricyanide/ferrocyanide and ferrous/ferric (claim 3), specifically ferricyanide/ferrocyanide (claim 4) whose concentration is within the range of 50  $\mu$ M to 1 mM (claim 5) and ratio is between 1:4 and 4:1 (claim 6). The methods of claim 2 is further specified to include the step of providing a second poisoning agent that is a reversible oxidation-reduction indicator (claim 7) that is selected from the group consisting essentially of methylene blue, toluidine blue, azure I and galloxyanine (claim 8) and adding at least two reagent mixtures (claim 11) comprising at least

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one growth supplement and one antibiotic supplement (claim 12). Similarly, the method of claim 1 is further specified to comprise adding at least two reagent mixtures (claim 9) comprising at least one growth supplement and one antibiotic supplement (claim 10).

The Office Action characterized Eden et al. as disclosing a method for detecting microbiological growth in a sealed sample chamber, in which the container headspace pressure is monitored as an indicator of microbiological growth (Abstract). Eden et al. do not disclose the inclusion of poisoning or redox buffering agents in the culture medium.

In Eden the presence of microbiological growth within the sealed sample chamber is detected by measuring the rate of change of headspace pressure in the container as the microorganism consumes oxygen and comparing the change in pressure to a reference standard of the initial pressure. A vacuum sensor senses a reduction in pressure in the headspace of a container and provides an electrical signal to remote electronics. A major problem exists for weak consumers, or slower growing organisms, where background redox reactions can occur because the reagents added to the culture broth cause an unpredictable change in the pressure differential in the headspace due to reduction oxidation. In other words, Eden is deficient in that it suffers from false positives due to background redox reactions.

The Office Action characterized Lancaster et al. as disclosing the use of poisoning agents or redox salts to inhibit autoreduction in microbial cultures without substantially affecting the desired reduction that takes place as a result of cellular metabolism [col. 3, 1.58 ff]. The addition of coupled pairs of reduced and oxidized salts or agents, such as ferricyanide/ferrocyanide and ferric/ferrous salts [col. 4, 1. 10 ff], with a concentration range of 50  $\mu$ M to 1 mM and a ferricyanide/ferrocyanide ratio of 1:4 to 4:1 [col. 7, 1. 15 ff], is preferred. In addition to the coupled poisoning agents, the growth media preferably includes a second poisoning agent that is also a reversible oxidation/reduction indicator. Suitable second poisoning agents include methylene blue (which has been found to stabilize the oxidation-reduction potential of the medium), toluidine blue, azure I and galloxyanide [col. 7, 1. 21 ff]. A suitable growth medium is used that

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may be supplemented with fetal calf serum, Hank's Modified Eagle Medium and Dulbecco's Modified Eagle Medium [col. 6, 1. 9 ff], and include antibiotics [Tables II and III].

The Office Action combined the two references based on the following reasoning and held claims 1-12 properly rejected under 35 U.S.C. § 103. In light of the preceding, one of ordinary skill in the art would have been motivated to make the substitution of the cell culture medium as described by Lancaster et al. [IDS] for that in the method of the primary reference (Eden et al. ) in order to obtain, with a reasonable expectation of success, the method as disclosed in the instant application. This would constitute the substitution of an art-accepted equivalent and have the obvious advantage of reducing oxidative stress on the micro-organisms being cultured. The claimed subject matter fails to patentably distinguish over the state of the art as represented by the cited references.

Applicant respectfully submits that the Lancaster et al. is directed to stabilizing the resazurin in the growth medium to inhibit autoreduction, to resorufin, a red-colored product which is the desired end point (emphasis added) when microbial or cellular growth is present. The resazurin will autoreduce in most or all growth media, where such autoreduction can cause a false change in color or fluorescence (emphasis added), both in the control and test samples. The incorporation of certain oxidation-reduction (redox) stabilizers, also referred to as poisoning agents, can substantially prevent autoreduction of the resazurin for extended time periods, usually for at least 24 hours or longer. Surprisingly, it has been found that the redox stabilizers can be added to the growth medium without substantially affecting the desired reduction which takes place as a result of cellular metabolism, even when the microorganisms or mammalian cells provide only weak metabolic reduction. See column 3, lines 46-63

Resazurin is also a pH indicator, having a blue color above a pH of about 6.5 to 6.8 and being red below that range of pH values. Column 8, lines 14-16

The visible light reading protocol used in accordance with this invention is based on the color

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shift from blue to red that is produced during the reduction of resazurin in the test well to resorufin as a result of microorganism growth. If there is growth of the microorganism in the test well 103, despite the presence of the preselected concentration of antimicrobial product disposed therein, then the test chemical solution present in the test well will turn from blue to red and a simple visual inspection of the test well provides a basis for determining a positive or negative test result. The growth control wells provide a basis for comparison of growth and no growth conditions to assist in identifying the condition of the test well. Column 8 lines 27-39

A particular advantage of the present invention lies in the ability to perform test protocols over extended periods of time. Since the stability of the resazurin in the control and negative test wells, as well as the resorufin in the positive test wells, is enhanced, the appearance of false negatives will be lessened or eliminated. Thus, additional incubation time for weakly growing organisms or cells can be provided to enhance the degree of color shift and facilitate recognition of positive test wells. Column 9, lines 44-52

Based on the above excerpted teachings from the Lancaster et al. patent, if there is growth of the microorganism in a test well, despite the presence of the preselected concentration of antimicrobial product disposed therein, then the test chemical solution present in the test well will turn from blue to red and a simple visual inspection of the test well provides a basis for determining a positive or negative test result. The Lancaster et al patent teaches that by incorporating certain oxidation-reduction (redox) stabilizers, also referred to as poisoning agents, the test chemical solution, autoreduction that can cause a false change in color or fluorescence (emphasis added), both in the control and test samples, is prevented. Since the stability of the resazurin in the control and negative test wells, as well as the resorufin in the positive test wells, is enhanced, the appearance of false negatives and resulting inaccurate conclusions will be lessened or eliminated.

Therefore, it would not be obvious to one skilled in the art to modify the teachings of the primary reference (Eden et al.) by the inclusion of poisoning or redox buffering agents in the



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culture medium as taught by Lancaster et al. In order to meet the terms of claim 1, there needs to be a teaching of providing a sealed sample container which contains a fluid mixture of a culture broth, the sample, and at least one poisoning agent for stabilizing the baseline pressure within a headspace above the fluid mixture in the sample container. Eden et al do not teach or suggest the inclusion of at least one poisoning agent for stabilizing the background noise within a headspace above the fluid mixture in the sample container, as noted in the Office Action. Lancaster et al do not teach or suggest the inclusion of at least one poisoning agent for stabilizing the baseline pressure within a headspace above the fluid mixture in the sample container. Instead, Lancaster teaches the inclusion of poisoning agents to reduce autoreduction that can cause a false change in color or fluorescence used to make conclusions as to the samples being tested. Therefore there is no reason, suggestion or motivation for the person of ordinary skill in the art to combine the teachings of Lancaster et al., i.e., to add a poisoning agent to a fluid mixture to stabilize the color of the mixture, to Eden et al where the pressure within the closed headspace above the mixture is being measured. Accordingly, claim 1 distinguishes over the applied art taken alone or in combination and therefore should be deemed allowable.

Claim 2 depends upon claim 1 and includes the step of providing a pair of coupled poisoning agents. Since none of the references taken alone or in combination teach or suggest using poisoning agents for stabilizing the baseline pressure within a headspace above the fluid mixture in the sample container, claim 2 should be allowable.

Claim 3 depends upon claim 2 and includes the step of selecting the pair of coupled poisoning agents from the group consisting essentially of ferricyanide/ferrocyanide and ferrous/ferric. Again, since none of the references taken alone or in combination teach or suggest using poisoning agents for stabilizing the baseline pressure within a headspace above the fluid mixture in the sample container, claim 3 should be allowable.

Claim 4 depends upon claim 3 and includes the step of selecting the pair of coupled poisoning agents is ferricyanide/ferrocyanide. For the reasons stated with regard to claim 3m claim 4

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should also be allowable.

Claims 5 and 6 depend upon claim 4 and should also be allowable.

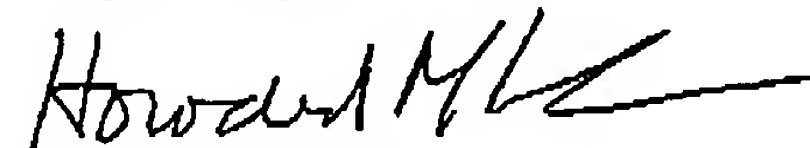
Claims 7 and 8 depend upon claim 2 and should also be allowable.

Claims 9 and 10 depend upon claim 1 and should also be allowable.

Claims 11 and 12 depend upon claim 7 and should also be allowable.

Applicant respectfully requests that a timely Notice of Allowance be issued in this case. No new matter is entered by this Amendment. If there are still some issues to be resolved, the Examiner is invited to contact the undersigned.

Respectfully submitted,



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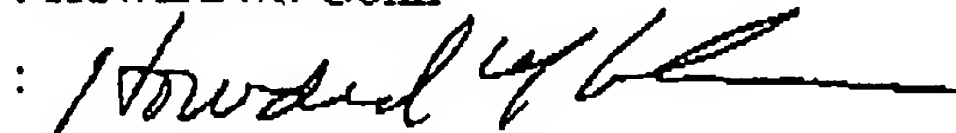
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